Preclinical Models of Wound Healing: Is Man the Model? Proceedings of the Wound Healing Society Symposium

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Abbreviations and Acronyms
RIN = RNA integrity number
WHS = Wound Healing Society

Significance: A review of therapeutic effects in preclinical and clinical studies suggests that concordance between large animal (pig = 78%), small laboratory animal (53%) and *in vitro* (57%) results with those observed in humans is only partial. Pig models of wound healing provide major advantages over other animal models. Since the vast majority of wound-healing research is done in rodents and *in vitro*, the low concordance rate is a significant impediment to research that will have any clinical impact.
Critical Issues: To generate clinically relevant experimental data, hypothesis generation should begin, or at least involve human wound tissue samples. Such tissue could be used to test a predetermined hypothesis generated based on, say, murine data. Alternatively, such tissue could be analyzed using high-throughput cell biology techniques (*e.g.*, genomics, proteomics, or metabolomics) to identify novel mechanisms involved in human wounds. Once the hypothesis has been formulated and confirmed using human samples, identification of these same mechanisms in animals represents a valid approach that could be used for more in-depth investigations and experimental manipulations not feasible with humans.
Future Directions: This consensus statement issued by the Wound Healing Society symposium strongly encourages all wound researchers to involve human wound tissue validation studies to make their animal and cell biology studies more translationally and clinically significant.

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The Wound Healing Society (WHS) sponsored a half-day preconference symposium on preclinical models of wound healing at the 2012 annual meeting in Atlanta. Under the directorship of Andrew Baird, PhD, and Gayle Gordillo, MD, the symposium explored the concept of using humans as a preclinical model for wound-healing research. A review of therapeutic effects in preclinical and clinical studies suggests that concordance between large animal (pig = 78%), small laboratory animal (53%), and *in vitro* (57%) results with those observed in humans is only partial.[1] Since the vast majority of wound healing research is done in rodents and *in vitro*, the low concordance rate is a significant impediment to research that will have any clinical impact. There is increasing pressure from NIH to demonstrate clinical significance to successfully compete for funding, which highlights the need to develop valid models that accurately represent the human clinical condition.

The introductory remarks made by the directors outlined the contextual framework of the symposium and put forth the concept that hypothesis generation should begin with human material. It could be used to test a predetermined hypothesis. Alternatively, it could be analyzed using high-throughput cell biology techniques, such as genomics, proteomics, or metabolomics, to identify novel biological or chemical events occurring in human wounds. Once the hypothesis has been formulated and confirmed using human samples, identification of these same biological or chemical events in animals would yield a valid model that could be used for more in-depth investigations and experimental manipulations not feasible with humans. The objective of the symposium was to stimulate investigators to use human subjects as the initial source material for hypothesis testing or generation to increase the clinical impact of wound-healing research.

Bob Diegelmann, PhD, was the first invited speaker of the symposium, and he began his talk by citing the Research Objectives of NIH RFA GM-06-002. He presented his work in trauma patients to develop an integrated system biology analysis of critical illness and injury. He inserts polytetrafluoroethylene tubes subcutaneously in trauma patients to sample the acute wound environment and analyzes the tissue that grows into these tubes with microscopy and high-throughput cell biology analysis or omics studies, including proteomics, metabolomics, lipidomics, and genomics. Thus, the entire spectrum of wound-healing processes and mechanisms can be analyzed and quantified in humans using state-of-the-art technologies.

Working directly with clinicians may not be possible for some investigators. To present an alternative method for accessing human samples, George Sandusky, DVM, PhD, Professor of Pathology and Lab Medicine at Indiana University, gave a talk on the fundamentals of biobanking. He noted improved sample preservation in liquid nitrogen compared to −80°C freezer and stressed the importance of sample quality assurance, which is typically done using two techniques. First is light microscopy to ensure the adequate sampling of the indicated tissue source. For example, tumor sections must consist of at least 65% tumor. Molecular quality control is determined by measuring the RNA integrity number (RIN). The interval between sample collection and fixation is a major determinant of the molecular integrity of the sample and ideally should be < 30 min. A RIN > 6 is considered acceptable for banked specimens.[3] He stressed the significance of robust data encryption to protect patient identities and the importance of including the ability to contact patients in the future as part of the Institutional Review Board protocol for the tissue bank.

The middle portion of the symposium was designed to discuss alternative strategies to increase the validity of the wound-healing research. It began with Elof Eriksson, MD, PhD, discussing alternative human models for wound-healing research. He urged members of the audience not to rely on models that have not been validated for the human condition even if they are well established in the literature. Boris Hinz, PhD, spoke about the importance of using *in vitro* models for conditions that are not well studied *in vivo* because of their complexity. He used mechanotransduction studies as an example. Only *in vitro* can dynamic cellular responses to mechanical stress be discriminated from cell responses to changes in their chemical environment. Susan Volk VMD, PhD, followed with a talk on the clinical veterinary experience of problematic wound healing in companion animals (dogs, cats, and horses), and how they may provide excellent opportunities for investigators as well. Because these veterinary patients are outbred with a relatively longer life, exposed to the same environmental factors as their human owners, they are commonly affected with similar comorbidities (such as diabetes and obesity). They often receive advanced clinical care similar to humans and may provide unique translational research opportunities. She highlighted the ample infrastructure to support research in vet schools, including clinical research centers and familiarity with NIH grants admin-

istration. She also noted lower regulatory hurdles to clinical testing in the veterinary market.

The last portion of the symposium used breakout groups to address obstacles and provide solutions to using human or animal materials for hypothesis generation. The biobanking group led by Dr. Sandusky and Stephanie Bernatchez, PhD, identified issues of scale with specimen repositories as an obstacle. Dr. Sandusky recommended using open source software developed by the National Cancer Institute called CaBIG and modifying it for the intended use of smaller groups of investigators. The financial realities of biobanking were also discussed, and Dr. Sandusky noted that 20% of the support the biobank at IU comes from grants, 55% from industry, and 25% from the University. He said a business plan and an appreciation of the complexities of biobanking were critical prerequisites to starting a biobanking program. Rob Kirsner, MD, and Marjana Tomic-Canic, PhD, led the group discussing team science. They identified funding, infrastructure, and a culture that bridges basic science and clinical practices as major obstacles. Their solutions included starting small and building up, sharing technologies, partnering with industry to support infrastructure development, and the importance of networking and communication. Luisa DiPietro, DDS, PhD, and Chandan K Sen, PhD, moderated a discussion on shaping scientific funding to facilitate wound-healing research with higher validity. They identified the scientific process and peer reviews as obstacles and said that investigators accepting the status quo were complicit in perpetuating science with a low clinical impact. They also identified a disconnection between NIH and the realities of the clinical importance of wound healing. Their solutions included branding wounds as a disease and getting a better mix of educated peer reviewers who put more emphasis on innovation and evidence-based translational medicine. They recommended building consortia between bench scientists and clinicians and partnering with industry to reduce reliance on NIH funding. Drs. Diegelmann and Eriksson moderated a discussion on how to get clinicians to work with you. Their recommendations included early identification and good communication among team members that should start at the time of protocol development. Dr. Boris Hinz, Laura Parnell, and Dr. Susan Volk moderated a large group discussing animal models, that divided into three subgroups to focus on acute wounds, chronic wounds, and scarring. Although true chronic wounds in laboratory animals do not exist, companion animals do develop chronic wounds, although with a low incidence. The swine models provide major strengths. Networking and multi-institutional studies to increase volumes of this patient population are necessary to complete translational studies. Acute wounds in rodent models heal quickly by contraction, so they recommended splinting wounds open to counteract this effect. When studying infected wounds, use of mixed cultures more accurately reflects the wound and microbial dynamics than a single organism. Large-animal models of excessive granulation tissue/hypertrophic scarring include the Red Duroc pig and limb wounds in horses, although limitations exist for both models. For the study of biofilms, pig models provide major advantages. Harriet Hopf, MD, WHS president, and Paul Liu, MD, WHS vice-president, moderated a discussion about how the WHS can facilitate the use of human preclinical source material. Their solutions were to use symposia, such as this one, to promote sharing of ideas, create a list serve for preclinical model, and a catalog/clearinghouse of standard operating procedures for preclinical models. They also recommended joining Researchgate, a social networking site for investigators.

> *"Over the years, basic and clinical research has revealed much about the individual molecular and cellular processes involved in wound healing, but attempts to accelerate and/or improve wound healing by enhancing, inhibiting, or modifying isolated aspects of the wound healing process have met with only limited success. Previous progress was hampered by the limitations of animal model systems in mimicking human wound healing, gaps in the understanding of how the molecular and cellular processes of wound healing are interconnected and interdependent."*
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> NIH RFA GM-06-002[2]

There were several important findings from this conference. As leaders in the field of wound healing, we must hold our peers accountable for producing research that is valid for the human or veterinary condition if that is the clinical endpoint. When other models are used, every effort should be made to validate those experimental models using actual human/veterinary wound tissue samples before committing to a specific line of investigation. Strategies for accessing human subjects or wound tissue samples were provided, including a review of the resources needed to support biobanking efforts. A concerted effort should be made to educate those who control funding allocation about the importance of this work.

REFERENCES

1. Sullivan TP, Eaglestein WH, Davis SC, and Mertz P: The pig as a model of human wound healing. Wound Repair Regen 2001; **9:** 66.
2. National Institutes of Health Centers for Innovative Wound Healing Research: Request for applications. Available online at http://grants.nih.gov/grants/guide/rfa-files/RFA-GM-06-002.html
3. Sandusky G, Dumaual C, Cheng L: Human tissues for discovery biomarker pharmaceutical research: the experience of the Indiana University Simon Cancer Center-Lilly Research Labs Tissue/Fluid BioBank. Vet Pathol 2009; **46:** 2.